ISSN (print) 0093-4666 © 2014. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/129.223

Volume 129(2), pp. 223–228 *October–December 2014*

Powdery mildew on *Bougainvillea spectabilis* in Mexico with a cryptic generic affinity

Sylvia P. Fernández-Pavía[1*], Gerardo Rodríguez-Alvarado[1], Jesús Gerónimo-Magaña[1], María Graciela Cabrera[2], Nuria Gómez-Dorantes[1], & Uwe Braun[3]

[1]*Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Km. 9.5 Carr. Morelia-Zinapécuaro, Tarímbaro, Michoacán 58880, México*

[2]*Universidad Nacional del Nordeste, Facultad de Ciencias Agrarias, Cátedra de Fitopatología, Sargento Cabral N° 2131, Corrientes 3400, Argentina*

[3]*Martin-Luther-Universität, Institut für Biologie, Bereich Gebotanik und Botanischer Garten, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany*

[*]Correspondence to: *fpavia@umich.mx*, *fernandezpavia@hotmail.com*

Abstract —An asexual powdery mildew on *Bougainvillea spectabilis* resembles *Ovulariopsis* (anamorphic *Phyllactinia* and *Pleochaeta*), while its combination of sinuous to coiled conidiophores forming dimorphic conidia singly suggests a *Pleochaeta* anamorph. However, this severely contradicts the rDNA ITS molecular data. The *Bougainvillea* powdery mildew shows the closest genetic similarities (80-83% sequence homologies) with various *Leveillula* and *Phyllactinia* species (*Phyllactinieae*), whereas *Pleochaeta* species were not genetically close. These results suggest that the *Bougainvillea* powdery mildew has an affinity with tribe *Phyllactinieae* of the *Erysiphaceae*, but the percentage of genetic similarity is below the scope of generic limits in powdery mildew ITS data. Although a new genus may be involved, we cannot formally propose it based on a single sequence, an anamorph morphologically indistinguishable from *Ovulariopsis,* and an unknown teleomorph.

Key words— *Erysiphales*, ornamental plant, new species, Michoacan

Introduction

Bougainvillea is a widely used ornamental plant in Mexico, with Morelos state one of the main producers in the country. In Michoacan it is the most common ornamental used in gardens, avenues, and parks. The flowers are also used for medicinal purposes, mainly as remedy for respiratory illnesses. The powdery mildew *Leveillula taurica* (Lév.) G. Arnaud has been associated with *Bougainvillea* in Mexico (Ramírez-Rojas et al. 2010), but its identification

has not yet been confirmed by molecular methods. The objective of this study was to determine the identity of the pathogen causing powdery mildew on 100–250 cm tall flowering *Bougainvillea spectabilis* plants collected during July and September 2009 in two urban municipalities in Michoacan, México. Some *Bougainvillea* plants showed chlorotic lesions on both sides of the leaves. Lesions were small (3–6 mm diameter), irregularly shaped, and with a central necrotic area. Most mature leaves of the affected plants showed lesions on the upper side of the leaves but lacked mycelia and other fungal reproductive structures. Lesions on the undersides were colonized by sparingly developed mycelia with few conidiophores and conidia.

Materials & methods

Two hundred forty-six *Bougainvillea spectabilis* plants were examined for powdery mildew in Morelia and Tarimbaro, municipalities in Michoacan state. Powdery mildew colonies on infected plants were prepared by means of a dissecting microscope and examined by fresh mounts in distilled water (with or without aniline blue) or dry mounts in 85% lactic acid, with staining and gentle heating (modified from Shin & La 1995 according to technique 5 in Liberato et al. 2005) and using standard light microscopy with oil immersion (bright field and phase contrast, Olympus BX 50, Hamburg, Germany). 30 measurements (1000× magnification) of conidia and other structures were made. Voucher specimens were conserved in the herbariums at Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico (EBUM) and Martin-Luther-Universität, Halle, Germany (HAL).

Genomic DNA was obtained from powdery mildew infected leaves from samples collected in gardens. Standard protocols followed instructions of the manufacturer (Wizard Genomic DNA Purification Kit, Promega Corp., Madison, Wisconsin). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA, including 5.8S rDNA, the 3' end of 18S rDNA and the 5' end of 28S rDNA, was amplified from powdery mildew according to Cunnington et al. (2003). PCR reactions were carried out using HotStarTaq DNA polymerase (HotStarTaq Master Mix Kit, Qiagen, Valencia, CA) in a thermal cycler (Mastercycler Gradient, Eppendorf). The PCR DNA products were cleaned using a commercial kit following the manufacturer's protocol (Wizard SV Gel and PCR Clean-up System (Promega, Corp., Madison, Wisconsin). PCR products were visualized by electrophoresis in 1.5% agarose gels in TAE buffer. Direct sequencing of the PCR products was carried out by an external company (Macrogen Inc. Seoul, Korea). Programs Pregap and Gap in the Staden Package (http://staden.sourceforge.net) were utilized to obtain consensus sequences for the amplified region from each isolate. Sequences were deposited in GenBank, 626 bp as *Ovulariopsis* sp. GRA-2013 (Accession no. KC556804). The sequences concerned were used in the BLASTN 2.2.25+ program (NCBI http://www.ncbi.nlm.nih.gov/) to determine the identity or at least the affinity based on sequence similarities expressed as percent sequence identity. A restricted phylogenetic tree was prepared, using the program Mega5 by neighbor-joining method with Tajima-Nei distance calculation to demonstrate relationships to the closest relatives.

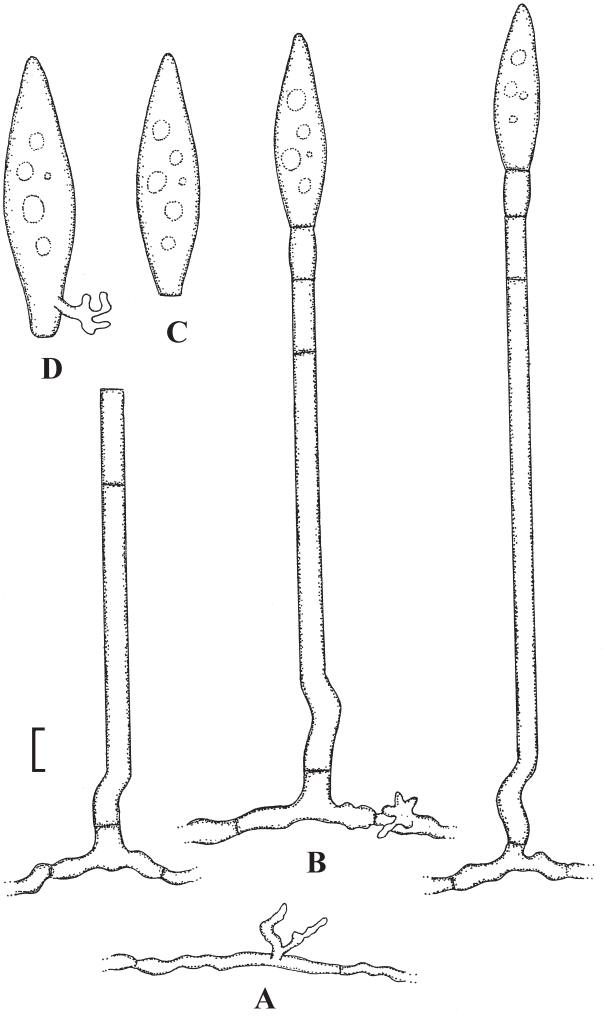

Figure 1. *Bougainvillea* powdery mildew.
A: Hypha with appressorium; **B**: Conidiophores; **C**: Conidium; **D**: Conidium with germ tube. Based on type material [HAL 2588 F]. Bar = 10 µm. U. Braun del.

Taxonomy

***Bougainvillea* powdery mildew** Fig. 1

Mycelium on leaves, hemiendophytic, superficial mycelium mostly hypophyllous, with hyphae emerging through stomata. Hyphae hyaline, septate, thin-walled, 2–6 µm wide, frequently with approximately right angled

branching. Hyphal appressoria numerous, distinct, oblong, hooked, sinuous and/or coral-like. Conidiophores consistently arising from the upper surfaces of superficial hyphae (conidiophores emerging through stomata not observed), and more or less midway between two septa, cylindrical, straight, unbranched, 140–252.5 (average 180) µm long and 7.5–9 µm wide, hyaline, thin-walled, smooth; foot-cells very long, cylindrical, but sinuous to somewhat coiled at the very base just above the basal septum that is slightly elevated above the junction with the mother cell, foot-cells followed by 1–2 much shorter cells. Conidia formed singly, one-celled, dimorphic, primary conidia lanceolate to ovoid-lanceolate, distinctly attenuated towards the apex, 62.5–87.5 (average 77.65) × 12.5–27.5 (average 20.85) µm, secondary conidia ellipsoid-subcylindrical, ends subtruncate, 55–77.5 × 7.5–15 µm, hyaline, with numerous oil-droplets, germ tubes arising near base or apex, single, short, producing hooked and/or coral-like appressoria.

Material examined: **MEXICO, Michoacan, Morelia municipality,** urban garden, on leaves of *Bougainvillea spectabilis* Willd. (*Nyctaginaceae*), September 2009, S. Fernández-Pavía (HAL 2588 F); September 2009, S. Fernández-Pavía (EBUM 23687, GenBank KC556804); **Tarímbaro municipality,** urban garden, on leaves of *B. spectabilis*, July 2009, S. Fernández-Pavía (EBUM 24362).

Discussion

The incidence of powdery mildew on *Bougainvillea* was very low: only four plants, or 1.6%, were infected. This is an atypical powdery mildew because all structures are only sparingly developed and rather inconspicuous, perhaps one reason that this fungus has been overlooked hitherto.

A blast search based on the sequence retrieved from our *Bougainvillea* powdery mildew (KC556804) did not yield any hits and showed only a distant similarity (80–83%) with other sequences representing *Leveillula* G. Arnaud and *Phyllactinia* Lév. These homologies are listed below:

83%: *Leveillula chrozophorae* U. Braun (AB045147, *Chrozophora tinctoria*, Iran), *L. duriaei* (Lév.) U. Braun (AB667859, *Phlomis persica*, Iran), and *L. taurica* s. lat. (AB667864, *Carthamus* sp., Iran).

82%: *L. duriaei* (AB044373, *Salvia nemorosa*, Iran), *L. elaeagni* (Jacz.) Simonyan & Heluta (AB042642, *Elaeagnus orientalis*, Turkmenistan), *L. lactucarum* Durrieu & Rostam (AB044375, *Lactuca serriola*, Iran), *L. lanuginosa* (Fuckel) Golovin (AB044376, AB045153, *Echinophora sibthorpiana* and *Heracleum persicum*, Iran), *L. loranthi* Haajian et al. (AB044377, *Loranthus europaeus*, Iran), *L. thevenotiae* (Jacz.) Golovin (AB044383, *Thevenotia persica*, Iran) as well as several sequences deposited as *L. taurica* s. lat. (AB045108, 667868, 667874, 667882, diverse hosts, Iran; AY912077, *Triglochin maritima*, origin unclear; GQ860947, *Mentzelia laevicaulis*, origin unclear; JQ885445, *Capsicum* sp., South Korea).

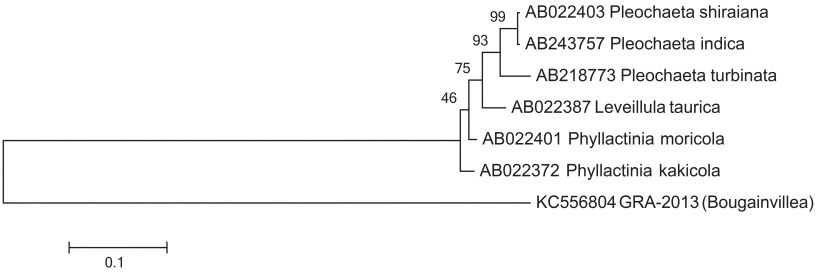

Figure 2. *Bougainvillea* powdery mildew. Restricted phylogenetic neighbor-joining tree, prepared by using the program Mega5 with Tajima-Nei distance calculation to demonstrate relationships of this powdery mildew to its closest relatives.

81%: *Phyllactinia fraxini* (DC.) Fuss (AB080513, *Fraxinus longicuspis*, Japan; AB080553, *F. excelsior*, Lithuania; AB080543, *Syringa vulgaris*, Germany).

80%: *P. roboris* (Gachet) S. Blumer (AB080516, *Castanea crenata*, Japan), *Phyllactinia* sp. (AB080546, *Castanopsis* sp., China; AB080564, *Chionanthus virginicus*, Germany).

No sequence agreeing with that of our *Bougainvillea* powdery mildew has yet been deposited in GenBank; our comparisons indicate that within powdery mildews the closest affinity is with species of the tribe *Phyllactinieae* R.T.A. Cook et al. Based on its most significant morphological characters — conidiophores that arise exclusively from superficial hyphae and with sinuous to somewhat coiled foot-cells — this fungus might be assigned to *Ovulariopsis* Pat. & Har., the anamorph of *Phyllactinia* and *Pleochaeta* Sacc. & Speg., as currently circumscribed by Braun & Cook (2012). The formation of dimorphic conidia rather suggest an asexual morph of *Pleochaeta* previously referred to as *Streptopodium* R.Y. Zheng & G.Q Chen, a genus originally introduced for *Ovulariopsis*-like powdery mildew anamorphs characterized by conidiophores with twisted foot-cells and associated with sexual morphs belonging to *Phyllactinia* and *Pleochaeta*.

Braun & Cook (2012) discussed asexual morphs of *Phyllactinia* and *Pleochaeta* in detail. Combinations of conidiophores with coiled foot-cells and formation of dimorphic conidia are not confined to *Pleochaeta* anamorphs, but are also known in various asexual morphs of *Phyllactinia* species, e.g., *P. bauhiniae* Y.S. Paul, *P. cassiae* G.J.M. Gorter & Eicker, and *P. chorisiae* Viégas. There is no real morphological basis for maintaining *Streptopodium* as a separate genus, and Braun & Cook (2012) reduced it to synonymy with *Ovulariopsis,* emending the circumscription of that genus. In addition, various sequences retrieved from

Pleochaeta species were not among those with highest homology (Fig. 2), in that *Pleochaeta* is more distantly related to the *Bougainvillea* powdery mildew than are *Phyllactinia* and *Leveillula*. The formation of dimorphic conidia and the conidial shape agrees well with species of *Oidiopsis* Scalia (anamorphs of *Leveillula*) but in *Leveillula* conidiophores emerge exclusively (or at least partly) through stomata and twisted conidiophore foot-cells are unknown.

In conclusion, we believe that the *Bougainvillea* powdery mildew undoubtedly represents an undescribed species related to genera of tribe *Phyllactinieae*. This powdery mildew is morphologically indistinguishable from the *Ovulariopsis* anamorphs of *Phyllactinia* and *Pleochaeta*, but it cannot be assigned to *Ovulariopsis* due to a conflicting molecular sequence analysis. This analysis confirms a distant relationship to *Leveillula*, *Phyllactinia*, *Pleochaeta*, and *Queirozia* Viégas & Cardoso but excludes it from being congeneric with any of these genera. The introduction of a new anamorph genus, based on molecular data of a single taxon and indistinguishable morphologically from *Ovulariopsis*, is not justified, as it would be of little help in clarifying the taxonomy of this powdery mildew. In the absence of an appropriate genus, it is currently not possible to provide a formal description of the *Bougainvillea* powdery mildew species. The discovery of the teleomorph of this mildew or additional genetically closely allied taxa would be useful, and so further research is necessary.

Acknowledgments

We are much obliged to Professor Xavier Madrigal Sánchez of Universidad Michoacana de San Nicolás de Hidalgo for support in the identification of the host plant, and we are thankful to the two reviewers, R.T.A. Cook (U.K.) and T.Z. Liu (Inner Mongolia, China), for critical comment.

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